Citation:

Mahon AK, Flynn MG, Stewart LK, McFarlin BK, Iglay HB, Mattes RD, Lyle RM, Considine RV, Campbell WW. Protein intake during energy restriction: effects on body composition and markers of metabolic and cardiovascular health in postmenopausal women.*J Am Coll Nutr*. 2007 Apr; 26 (2): 182-189.

PubMed ID: 17536130

Study Design:

Randomized controlled study.

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

POSITIVE: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

- The main purpose of the study was to compare short-term effects of two energy-restricted, moderately high-protein diets (different protein source) with an ovolactovegetarian lower-protein diet
- The secondary purpose was an analysis of lipoprotein-lipid profile, C-reactive protein, glucose, insulin, leptin and adiponectin concentrations in overweight and mildly obese pre-menopausal women.

Inclusion Criteria:

- *Inferred:*
  - Female
  - Age at least 50 years old, but less than 80 years old;
  - At least two years post-menopausal
  - Body mass index (BMI) greater than 25kg/m^2 but less than 35kg/m^2
  - Nonsmoker
  - Normal kidney and heart functions
  - Non-diabetic; stable thyroid disease, etc.
- *Stated:* Medical and diet histories that included
  - Height
  - Weight
  - Blood pressure
  - Cardiologist-interpreted resting electrocardiogram
  - Clinical urine and blood chemistries.

Exclusion Criteria:

- Male

- Age less than 50 years or more than 80 years
- Less than two years post-menopausal
- BMI less than 25kg/m^2 or more than 35kg/m^2 at the time of screening
- Smoker
- Clinically abnormal kidney, liver or heart functions
- Disease conditions such as diabetes, unstable thyroid disease
- Have abnormal protein or hematological status
- Receiving insulin replacement therapy or anti-inflammatory steroid medications.

Description of Study Protocol:

Recruitment

- Newspaper ads and community postings
- Stipend provided.

Design

Randomized controlled trial.

Intervention

- Nine-week dietary intervention: One of three diets were assigned
- Total intakes of 1,250kcals (1,000 basal and 250kcal from beef, or chicken or carbohydrate)
- This basal diet was distributed as a five-day fixed menu that consisted of three meals and two snacks equaling 1,000kcals
- Groups were distinguished by source of protein as described below:
    - Beef group was provided with 250kcal per day of cooked beef
    - Chicken group was given 250kcal per day of cooked chicken breast and 10g butter
    - Ovolactovegetarian group was provided with shortbread cookies and sugar-coated chocolates (lower protein, 10% of energy) and considered the CARB group.
- Limited substitutions were allowed: Vegetables and non-vegetable items (salad dressings, decaffeinated beverages and water)
- Each participant received individualized dietary counseling two times per week
- Energy and macronutrient intakes were assessed from three-day food records
- Habitual activities were encouraged; new structured exercise regimens were discouraged
- Urine and blood collection using standard protocols
- Body composition assessment taken pre-intervention and post-intervention for estimation of percentage of body fat, fat mass and fat-free mass using DEXA
- Physical activity survey administered pre-intervention and post-intervention to estimate energy expenditure.

Statistical Analysis

- Table of random numbers were used for assignment of participants
- One-factor ANOVA used for pre-assessment of diet
- A four-times-two ANOVA table was used with repeated measures
- Tukey's post-hoc analyses were used to determine group differences.

Special Diet Prescriptions or Modifications
(e.g., low fat, etc.)

Nutrients	Ovolactovegetarian	Beef Diet	Chicken Diet	Control
Energy	1,250kcal	1,250kcal	1,250kcal	Habitual diet
Protien	50g (16%)	80g (26%)	80g (26%)	NR
Carbohydrates	180g (58%)	150g (48%)	150g (48%)	NR
Fat or Fatty Acids	27g (26%)	27g (26%)	27g (26%)	NR
Other: Saturated Fat	10%	10%	11%	NR
Supplements				NR

*Calcium was permitted with physician's recommendation for all groups.

- *Length of treatment:* 11 weeks (nine-week intervention and two-week weight maintenance period)
- *Follow-up:* Individual counseling two times each week for nine weeks; non-fasting total body mass index (BMI) was measured twice per week; energy and macronutrient intakes estimated from three-day food records
- *Behavioral or educational interventions:* Individual counseling twice per week.

Data Collection Summary:

Timing of Measurements

Nine weeks of intervention.

Dependent Variables

- *Variable One:* Weight loss and body composition (total body mass, percentage of body fat, fat mass and fat-free mass; measured using DEXA)
- *Variable Two:* Lipid lipoprotein profile and other metabolic markers measured through blood collection taken between 7:00 a.m. and 9:00 a.m. after a 10-hour overnight fast (venous blood)
- *Variable Three:* Markers of dietary protein intake using 24-hour urine collection (pre- and post-).

Independent Variables

Energy-restricted diet: 1,250kcal per day; consisted of ovolactovegetarian (CARB) group, Beef and Chicken Groups (moderately high protein from different sources).

Control Variables

Habitual diet (CON group).

Description of Actual Data Sample:

- *Initial N:* 61 post-menopausal women
- *Attrition (final N):* 54
- *Age:* Greater than or equal to 50 years, but less than 80 years
- *Ethnicity:* Caucasian.

Other Relevant Demographics

Metabolic markers, pre-intervention

- *Cholesterol:* 248±60mg per dL
- *LDL:* 153±47mg per dL
- *Prediabetic:*
  - FPG: 100±13mg per dL
  - Normo insulinemic: HOMA 0.39±0.2
- *CRP:* Borderline elevated (2.9±3.4mg per L).

Anthropometrics: Body Composition

- *BMI:* At pre-intervention, they were 30.1kg/m^2 (Beef Group); 29.1kg/m^2 (Chicken Group); 28.4kg/m^2 (CARB Group) and 30.1kg/m^2 (Control Group)
- *FFM:* At pre-intervention, they were 45.5kg (Beef Group); 43.3kg (Chicken Group); 42.4kg (CARB Group) and 44.5kg (Control Group)
- *Percentage of body fat:* 43.4% (Beef Group), 42.9% (Chicken Group), 43.7% (CARB Group) and 44.9% (Control Group)
- *Fat mass:* 35.4kg (Beef Group), 32.9kg (Chicken Group), 33.5kg (CARB group) and 35.3kg (Control Group)
- *Body mass:* 81.0kg (Beef Group), 76.2kg (Chicken Group), 75.9kg (CARB group) and 79.8kg (Control Group).

Location

Greater Lafayette, Indiana, US.

Summary of Results:

Dietary Intakes

Energy and Nutrients	Control *Pre* *(Post)*	BEEF *Pre* *(Post)*	Chicken *Pre* *(Post)*	Vegetarian/CARB *Pre* *(Post)*
Energy (kcal per day)	1,698±488 (1,570±633)	1,862±653 (1,114±155)	1,579±487 (1,098±203)	1,699±468 (1,158±341)
Protein (grams per day)	71±24 (70±41)	80±33 (67±9)	68±25 (67±12)	75±20 (51±21)
Carbohydrates (percentage of kcal)	49±5 (47±3)	45±8 (46±7)	48±12 (51±5)	49±7 (59±8)
Total Fiber (grams per day)	18±8 (14±8)	17±7 (16±5)	15±8 (17±5)	16±5 (18±8)
Fat (percentage of kcal)	36±6 (33±6)	38±8 (30±5)	36±9 (24±4)	34±6 (24±6)

Intake

- Energy and macronutrient intakes were lower among intervention diet groups versus

controls. However, the three intervention diet groups did not differ significantly from each other.
- The percent energy intake from protein was lower in the Vegetarian group compared to Beef, Chicken and Control groups.

Other Findings

- Marker of protein intake: Change in nitrogen excretion from PRE to POST was different (group by time, P<0.001) for CARB (baseline, 7.6±4.0g per kg per day; post, 4.8±1.3g per kg per day) compared to Beef (baseline, 7.5±2.4g per kg per day; post, 8.6±3.3g per kg per day), Chicken (baseline, 7.7± 2.3g per kg per day; post, 8.3±1.3g per kg per day) and CON (baseline 8.1±1.8g per kg per day; post, 7.8±2.2g per kg per day)
- Physical activity report showed no differences over time or among groups.

Body Composition, CVD Risk Factors and Metabolic Markers

Variables		**Control Baseline** *(Change at Nine Weeks)*	**Veg/CARB, Baseline** *(Change at Nine Weeks)*	**Beef Diet, Baseline** *(Change at Nine Weeks)*	**Chicken Baseline** *(Change at Nine Weeks)*	**Statistical Significance of Difference Diets**
Blood Lipids	*Total Cholesterol (mg per dL)*	300±70 (-6±56)	284±87 (-44±66)	241±57 (-23±36)	218±37 (-19±48)	All group change, P=0.003
	HDL (mg per dL)	68±15 (3±15)	73±19 (-12±17)	59±15 (-2±11)	50±10 (-0±16)	NS
	LDL (mg per dL)	184±32 (-10±46)	161±52 (-20±50)	157±49 (-17±27)	141±40 (-16±45)	All group change, P=0.013
	TC/HDL ratio	-0.3±0.2	0.1±0.3	-0.5±0.1	-0.3±0.3	NS
	Triacylglycerol (mg per dL)	156±46 (-2±58)	183±95 (-10±69)	127±57 (-23±50)	139±57 (-25±45)	NS
	CRP (mg per L)	3.5±3.7 (-0.3±1.1)	3.8±6.1 (-0.6±4.0)	2.4±2.1 (-0.0±1.5)	2.6±3.6 (-0.4±2.1)	NS
Body Composition	*Body Mass (kg)*	79.8±11.4 (-1.2±1.2)	75.9±8.8 (-5.6±1.8)	81±9.2 (-6.6±2.7)	76.2±11.0 (-7.9±2.6)	Intervention groups vs. control, P<0.05 Veg vs. Chicken, P<0.05

	BMI (kg/m^2)	30.1±3.8 (-0.3±0.5)	28.4±3.3 (-2.1±0.7)	30.1±3.1 (-2.5±1.1)	29.1±4.3 (-3.0±1.2)	Intervention groups vs. control, P<0.05 Veg vs. Chicken, P<0.05
	Fat Mass (kg)	35.3±9.5 (0.6±3.8)	33.5±7.3 (-3.9±1.5)	35.4±7.7 (-4.3±2.1)	32.9±7.3 (-5.6±2.2)	Intervention groups vs. control, P<0.05
	Percentage Body Fat (%)	44.9±6.7 (-0.2±1.2)	43.7±5.1 (-2.1±1.5)	43.4±5.1 (-2.1±1.8)	42.9±4.1 (-3.3±1.7)	Intervention groups vs. control, P<0.05
	Fat-free Mass (kg)	44.5±3.4 (0.0±1.0)	42.4±3.0 (-1.7±1.0)	45.5±3.4 (-2.2±1.3)	43.3±4.6 (-2.3±1.0)	Intervention groups vs. control, P<0.05

Body Composition Outcomes

- All three diet intervention groups significantly improved body composition measures relative to the control group
- There were no significant differences in changes of body composition measures among intervention diet groups except the Chicken group lost significantly more weight and reduced BMI more than the Vegetarian group.

Cardiovascular Disease Outcomes

- Even though total and LDL cholesterol decreased across all groups, there were no significant changes among all diet groups and control for these values
- There were no significant changes for any of the groups in Triacylglycerol, HDL cholesterol, or C-reactive protein, and there were no significant differences among groups.

Metabolic Markers

Metabolic markers did not change significantly over time for any of the groups, nor was there significant difference among groups. The following metabolic markers remained unchanged.

- Fasting glucose
- Insulin
- Adiponectin
- Leptin
- Insulin sensitivity (HOMA).

Change in insulin was not correlated to change in weight.

Author Explanation of Lack of Findings

- The authors say that the lack of significant changes for insulin sensitivity and C-reactive protein could be due to low sample size. The lack of significant difference between the Vegetarian and Beef groups for weight (body mass) could also be due to the small sample size.
- Differences between the Chicken and Vegetarian groups in terms of weight loss and BMI cannot be attributed to protein source.

Author Conclusion:

Findings support that overweight post-menopausal women can lose weight and improve lipid-lipoprotein profile using a moderate-protein (25% energy intake) of beef, poultry or a lower protein (17% energy intake) or a ovolactovegetarian diet.

Reviewer Comments:

- *Strengths: Randomized controlled study; diets were similar for energy, fat, protein and fiber across treatment groups*
- *Concerns: Could have provided a bit more on statistical analyses in the results*
- *Limitations: Short period of intervention so it was not possible to observe relevant changes due to source of protein; trans fat was not a consideration for lipid profile*

Overall a good study, need replication with longer intervention and a bit more structure on the treatment groups (especially vegetarian).

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions

1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions

1.	**Was the research question clearly stated?**	Yes

1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes

4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	Yes
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	Yes
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	Yes
6.6.	Were extra or unplanned treatments described?	No
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes

7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	No
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	No
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	No
8.6.	Was clinical significance as well as statistical significance reported?	No
8.7.	If negative findings, was a power calculation reported to address type 2 error?	Yes
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes